J. Egypt. Soc. Toxicol.
(Vol. 34: 71-75 Jan. 2006)
WWW.estoxicology.org

ALTERATION IN ERYTHROCYTE MEMBRANE FATTY ACIDS COMPOSITION, CHOLESTEROL AND PHOSPHOLIPIDS OF CHRONIC LEAD INTOXICATED MALE RABBITS AND THEIR MODULATION WITH VITAMIN E

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ABSTRACT

Membrane lipids specially fatty acids appear to be one of the critical target associated with lead intoxication. The aim of our research was to elucidate the changes in erythrocyte membrane fatty acids composition, cholesterol and phospholipids (that may affect different aspect of cell structure and function) under the effect of chronic lead toxicity and their modulation with vitamin E. For this purpose forty two male new-Zealand rabbits of 2-3 months old, average body weight 2-3 kg were randomly divided into three equal groups, each of 14 animals. The first group (GI) kept as control. The second group (G II) received orally lead acetate (l/20 of L.D.50) daily for 90 days. The third group (G III) administrated orally (lead acetate + vitamin E) daily for 90 days. Heparinized blood samples were obtained from all groups, plasma was separated and used for determination of lipid peroxidation (L. malondialdehyde). Red blood cells were processed for determination of cholesterol, phospholipids and fatty acid composition in erythrocytes membrane. Our data revealed significant increase in plasma MDA level ($P < 0.001$) and erythrocyte membrane cholesterol ($P < 0.05$) in lead intoxicated group, which decreased after vitamine E treatment. Whereas a significant decrease in membrane phospholipids ($P < 0.05$) and alteration in fatty acid pattern which also modulated by vitamin E supplementation..

Keywords: **Erythrocyte Membrane – Fatty Acids – Cholesterol – Phospholipids – Vitamin E – Lead - Rabbits**

INTRODUCTION

Lead is one of the most commonly encountered toxic elements Although many countries now have programs to lower the level of lead (Pb) in the environment, human exposure to it remains the concern to public health officials worldwide (Zhao *et al.*, 2004). As it remains biologically active long after its release to environment and the stability of its compounds prevents its degradation, as well as wide spread industrial use has made lead as serious threat to human and animal health (Irena and Alina 2004).

More than 99% of lead present in circulatory system is bound to the erythrocytes 80% of which located in cytoplasm and 20% being found in the cell membrane (Mishra *et al.*, 2003) specially membrane lipids which not only play a structural role but also constitute an element of metabolic process, as there is a close correlation between the function of proteins and the state of lipid phase (Przestalski *et al.*, 2000).

Very little information have been obtained regarding lead effect on erythrocytes cell membrane specially membrane lipids.

So the present study aimed to investigate the harmful effect of lead on the erythrocyte membrane fatty acid composition, cholesterol, phospholipids and plasma lipid peroxidation in male rabbits as well as the possible protective effect of vitamin E.

MATERIAL AND METHODS

Forty two white male New-Zealand rabbits of 2-3 months old, average body weight 2-3 kg were used in this study. The rabbits were randomly divided into three equal groups each of 14 animals.

Group I: Kept as control.

Group II: Received lead acetate (1/20 of LD_{50}) daily at a dose of 19.5 mg/kg.b.w. orally for 90 days.

Group III: Adminstrated (lead acetate + vitamin E) daily at a dose of 19.5 mg/kg.b.w. and 18.67 mg/kg.b.w. orally for 90 days, doses were calculated according to Pagget and Barnes (1964).

Heparinized blood samples were obtained from all animals, plasma was separated from erythrocytes and used for determination of lipid peroxidation (L. malondialdehyd), using method adapted by Esterbauer *et al.* (1982).

For determination of cholesterol, phospholipids and fatty acid composition in erythrocytes membrane, pcked cell were washed three times with an equal volume of phosphate buffer pH 7.4, the supernatant solution was removed by aspiration together with 2-3 mm of p.c.v. containing white blood cell. The washed RBC's were suspended in NaCl 0.9% to provide 50% hematocrite, then heamolyzed by placing 200 µl of the erythrocyte suspension at -20°C for 2 hour and at 37°C for 5 min (Peuchant *et al.*, 1989)

Lipid extraction: Using isopropanol and sufficient anhydrous sodium sulfate, after centrifugation aliquots were used for cholesterol determination using the method of Allain *et al.* (1974), and phospholipids were determined according to Takeyama (1977).

Fatty acid ester preparation and methylation of fatty acids according to Vogel (1975). The methyl ester of the fatty acids and standard compounds were analyzed using Pye unicamm pro G.C.sp 2300 gas chromatograph equipped with dual flame ionization detector at Centeral Laboratory, Faculty of Agriculture Moshtohor, Benha University.

Statistical analysis of the obtained data was carried out using t test according to Kempthorn (1969).

RESULTS

Table (1) showed significant decrease in erythrocyte membrane phospholipids of lead intoxicated rabbit while cholesterol concentration was significantly increased compared with control group.

Moreover, a highly significant increase in plasma malon-dialdehyde level was demonstrated in lead intoxicated rabbit compared to normal group.

Treatment of lead exposed rabbits with Vit. E significantly decreased the level of malondialdehyde compared with lead intoxicated group also ameliorate the changes in erythrocyte membrane phospholipids level.

Table (2): revealed marked increase in erythrocyte membrane saturated fatty acids especially stearic and arachidic which showed significant increase in chronic lead intoxicated group compared with control one, while vitamin E treated group recorded significant decrease compared to lead intoxicated group. A significant decrease in the proportion of polyunsaturated fatty acids (P.U.F.A.) especially linolenic acid which showed a highly significant decrease in lead intoxicated group compared with control group, this decrease changed to highly significant increase in vitamin E treated group compared with lead intoxicated rabbit. Monounsaturated fatty acids showed a non significant changes.

Table (1): Mean value of erythrocyte membrane phospholipids, cholesterol and plasma malondialdehyde level after three months of lead toxicity and vitamin E treatment of male rabbits.

Parameter	Group I	Group II	Group III
Phospholipids (µmol/10^{11} RBC)	17.11+0.57	12.81+0.78*	15.97+0.47#
Cholesterol (µmol/10^{11} RBC)	16.75+0.51	19.92+0.69*	18.29+0.96
Malondialdehyde (n mol/ml)	4.25+0.28	7.93+0.46**	5.16+0.62#

* Significant different from G1 at (P < 0.05). # Significant different from GII at (P < 0.05). ** Significant different from GI at (P < 0.01).

Table (2): The major fatty acid composition and the main change in erythrocyte membrane fatty acid pattern after three months of chronic lead toxicity and treatment with vitamin E in male rabbit.

Fatty acid %	Group I	Group II	Group III
Caproic 10:0	4.00+0.56	N.D.	6.42+0.88
Doaecanoic (Lauric 12:0)	11.30+1.05	N.D.	12.29+1.19
Tetradecanoic (Myristic 14:0)	5.13+0.58	6.97+0.43	4.99+0.69
Unknown	4.37+0.75	3.90+0.49	5.73+0.58
Hexadecanoic (Palmitic 16:0)	11.84+1.23	12.65+1.06	9.15+0.76
Unknown	5.25+0.68	3.67+0.42	4.34+0.58
Octadecanoic (Stearic 18:0)	32.30+2.01	44.78+3.41*	30.23+1.91#
Octadecanoic (Oleic 18:1) Δ 9	7.00+0.55	8.50+0.76	6.85+0.85
Octadecanoic (Linoleic 18:2) Δ 9, 12	9.03+0.73	8.19+0.52	10.80+0.71#
Octadecatrenoic (Linolenic 18:3) Δ 9, 12, 15	2.50+0.42	0.51+0.08**	5.36+1.13##
Eicosaenoic (Arachidic 20:0)	5.61+0.65	10.38+1.63*	3.76+0.68#
Total saturated fatty acids (T. SFA)	63.01+1.95	73.88+3.39*	61.14+2.02#
Total monounsaturated fatty acids (T. MUFA)	7.00+0.55	8.50+0.76	6.85+0.85
Total poly unsaturated fatty acids (PUFA)	13.53+1.04	8.69+0.89*	16.16+1.45##

Δ Position of double bonds counted from COOH end. N.D.: Not detected

* Significant different from GI at (P < 0.05). ** Significant different from GI at (P < 0.01).

Significant different from GII at (P < 0.05). ## Significant different from GII at (P<0.01).

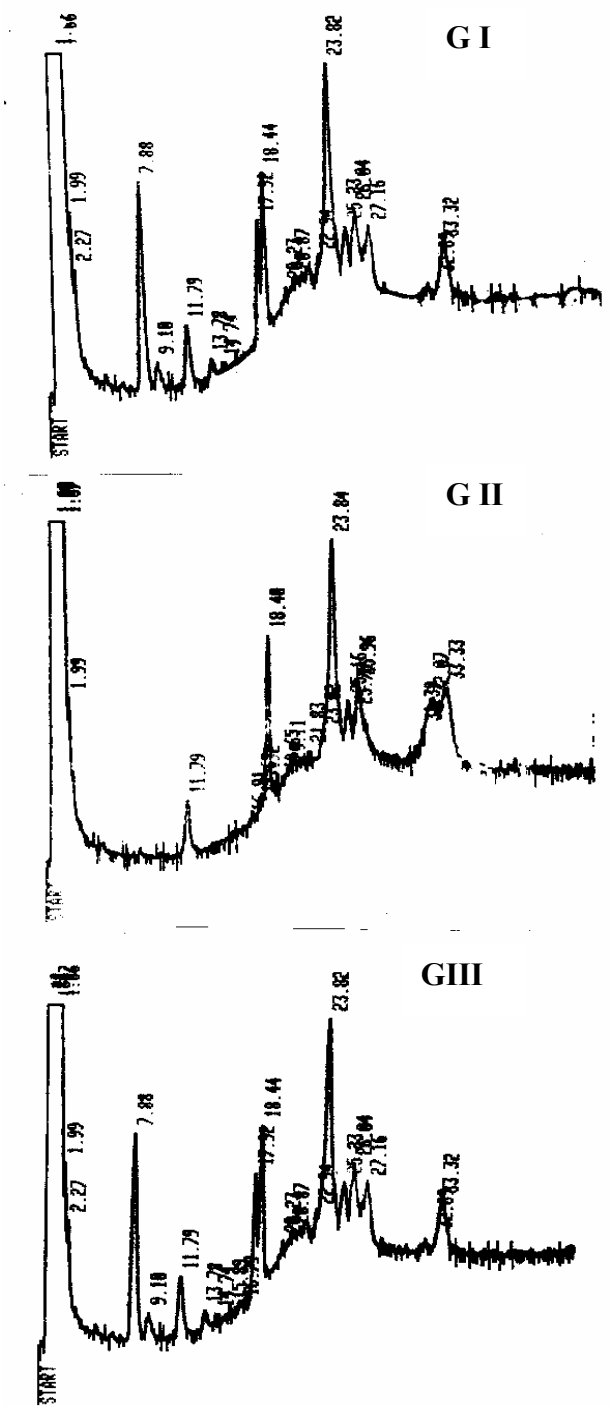

Fig. (1): Gas chromatogram of erythrocyte membrane fatty acid pattern of normal control male rabbit (GI), chronic lead intoxicated male rabbit (GII) and chronic lead intoxicated and vitamin E treated male rabbit (GIII).

DISCUSSION

In lead toxicity the production of free radical is accelerated due to abnormal metabolic regulation which resulted in oxidative damage (Kawage *et al.*, 2001). The recorded significant increase in plasma malondialdehyde concentration of lead intoxicated rabbit came in accordance with the data obtained by Mohamed (2004) who explained this result as, the system of free radical generation include xanthin oxidase and phospholipase A2 activation which hydrolyze membrane phospholipids leading to decrease in phospholipids and increase the concentration of lipid peroxide (Douillent *et al.*, 1998). In addition, animal subjected to lead intoxication showed decline in the thiol capacity of the cell as lead binds sulphadryl group and interfere with SH containing enzymes such as gluthathione reductase leading to reduction in glutathione stability, which increase succeptibility to lipid peroxidation (Sivaprased *et al.*, 2002). The observed significant decrease in erythrocyte membrane phospholipids of lead intoxicated rabbit may be due to increased generation of reactive oxygen species which are responsible for oxidative injury to erythrocyte membrane, whereas the ratio between GSH and GSSG was reduced (Cazzola *et al.*, 2004). Moreover lead particles adhered to the external and internal surface of erythrocyte membrane, induced molecular disorder in both lipid bilayer (Sawalsky *et al.*, 2003) via activation of scramblase, leading to phosphatidylserine exposure (Kempe *et al.*, 2005). As well as any alteration in membrane lipids leads to change in membrane fluidity which in turn alter ATPase activities since the membrane bound enzymes are SH containing which are lipid dependent, leading to change in the level of membrane cholesterol and phospholipids (Upasani *et al.*, 2001). Concerning the effect of vitamin E. Our results were similar to those mentioned by Rhee *et al.* (1995), where dietary supplementation of Vit. E inhibit phospholipase A2 activity resulted in decreases in lipid peroxide. Thus it was suggested that, antioxidant such as Vit. E might play an important role in the treatment of lead poisoning (Chen *et al.*, 2002), it can maintain the physical properties of membrane associated with enzyme activities and modulate phospholipids and cholesterol content, contributing to cholesterol haemostasis (Douillet and Ciavatti, 1995). This suggestion was supported by the finding of Upasani and Balaraman (2001) who recorded that the level ATPase has been restored to near normal in Vit. E treated animal, attributed these results to the ability of Vit. E to protect the sulphadryl group from oxidative damage through inhibition of peroxidation of membrane lipids.

Regarding erythrocyte membrane fatty acid pattern. It was clearly observed that lead intoxication exhibits some picular changes in fatty acid represented as significant increase in saturated fatty acid mainly stearic (18:0) and arachidic (20:0) that may be due to increase in oxidation than esterification (Mohamed, 2004) as lead compete with zinc in some enzyme such as Cu/Zn superoxid dismutase leading to accumulation of superoxid radicals (Oswellers,

1996), also activate phospholipase A_2 that attack the ester link of β position of phospholipids liberating free fatty acids and lysophosphatides (Brin *et al.*, 1974).

On the other hand, the recorded significant decrease of PUFA in lead intoxicated group could be related to the increased ratio between pro-oxidant and antioxidant by lead via its adherence in erythrocyte membrane and increasing of reactive oxygen species (ROS) which decreased the ratio between reduced and oxidized glutathione (Cazzola *et al.*, 2004).

This opinion is confirmed by Vao *et al.* (1994) as the double bonds of PUFA were attacked by the oxygen and hydroxyl radicls forming peroxyls which had damaging effect on cell membrane. Vitamin E is a hundred of time more potent as hydrogen donners than other aromatic antioxidant, so it was a cytoprotective against variety of toxic agents (Wang *et al.*, 1996), it can interrupt the chain reaction via conversion of the PUFA peroxy radical to its hydroperoxid form that prevent inflecting damage on neighboring poly unsaturated fatty acids.

Conclusion:

It could be concluded that, lead ion alter erythrocyte membrane fatty acid composition, cholesterol and phospholipids resulted in decreased membrane resistance to oxidation and finally erythrocyte fragility leading to destruction, while vitamin E protect erythrocyte membrane lipids against peroxidation and damage caused by chronic lead toxicity.

We recommend that administration of vitamin E even from natural sources can be beneficial for human at risk for lead intoxication as workers and children.

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